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A NOTE ON HYDROCYANIC ACID IN THE BURMA BEAN (*PHASEOLUS LUNATUS* SP.)

BY

F. J. WARTH, B.Sc., M.Sc

Physiological Chemist, Pusa

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PREFACE

Owing to my transfer from Burma to my present post I was unable to carry out the programme of work I had undertaken. The results obtained up to the date of my departure are recorded in this note in the hope that they may be of assistance in the further prosecution of the work.

F. J. Warth.

A NOTE ON HYDROCYANIC ACID IN THE BURMA BEAN (*PHASEOLUS LUNATUS* SP.).

BY

F. J. WARTH, B.Sc., M.Sc.,

Physiological Chemist, Pusa.

[Received for publication on the 12th July, 1922.]

I. The determination of HCN.

THAT the determination of HCN in plant products is not a straightforward matter may be gathered from the voluminous literature on this subject. Two examples will suffice to show the nature of the difficulties which may be met with. Alsberg and Black (*Jour. Biol. Chem.*, 1916) found that leaves of *Prunus Virginiana* must be distilled with acid for four hours in order to liberate all the HCN, whereas one hour is sufficient for *Andropogon*. They found also that by macerating plant tissues containing HCN a certain amount of the HCN present or of cyanide added is so converted that it cannot be recovered by distillation with H_2SO_4. They considered this was not due either to enzymes or to the presence of glucose. They recommended that in determining HCN in plants several methods should be employed to corroborate one another. Viehover Johns and Alsberg (*Intern. Bull.*, 1918) working with *Tridens flavus* found that maceration followed by distillation with acid gave lower results than immediate distillation with acid. Distillation of the macerated or unmacerated plant without acid resulted in a partial or complete loss of the available HCN.

In my experiments with the Burma bean macerated and unmacerated leaf gave identical results. Acid also gave no increased yield in conjunction with the enzyme process. The procedure to be adopted must, therefore, be varied according to the plant under investigation.

The determination of HCN in the Burma bean offers its own peculiar difficulties however; but before entering into these, the analytical methods employed will be briefly outlined.

1. *Auto-enzyme hydrolysis.* The material—macerated or ground if this preliminary treatment appeared to be necessary—was soaked in water for 24 hours generally. The HCN liberated by this treatment was distilled off and absorbed in alkali. The distillation was usually effected by means of steam and the HCN determined as a rule by precipitation as Prussian blue.

2. *Extraction and separation of the crude glucoside with alcohol and its subsequent hydrolysis with* H_2SO_4. This process was carried out on the lines laid down by Dunstan and Henry.

3. *Extraction with hot water.* For Burma beans I have found that extraction with boiling water is as effective as and more convenient than the alcohol extraction. The third method therefore consisted in extracting the glucoside with boiling water and hydrolysing the extract with acid. In this process it must be noted that when dealing with material containing active enzyme, such as the leaf, some liberation of HCN is to be expected at the moment of plunging the leaf into boiling water. This was found to be the case very frequently.

The HCN so formed (it will be spoken of as free HCN for the sake of brevity) had to be distilled off and determined separately before proceeding with the extraction and hydrolysis. In the water extraction process generally 2 figures will be given, namely :—

(*a*) Free HCN.

(*b*) HCN by acid hydrolysis.

It should be understood that these two figures combined give the HCN obtainable by this method. The free HCN is, however, generally negligible in amount.

As the acid hydrolysis was required for both the second and third processes it has been thoroughly tested to arrive at the best conditions of working.

The following procedure was finally adopted as yielding the highest results in the time available for completing the determination in one day.

The extract made up to 300 c.c., containing 50 c.c. conc. H_2SO_4, was placed in a flask and connected to a condenser at the delivery-end of which an alkali

scrubber was attached. Hydrolysis was allowed to proceed by heating the flask in a vigorously boiling water bath. At the end of two hours and again at the end of four hours a rapid current of steam was passed through the hydrolysing liquor to remove all free HCN to the absorbing bottle. The hydrolysis was stopped at the end of six hours and a final passage of steam employed to remove the last traces of HCN.

The graph (Fig. 1) shows that by this procedure practically all the determinable HCN can be liberated in six hours.

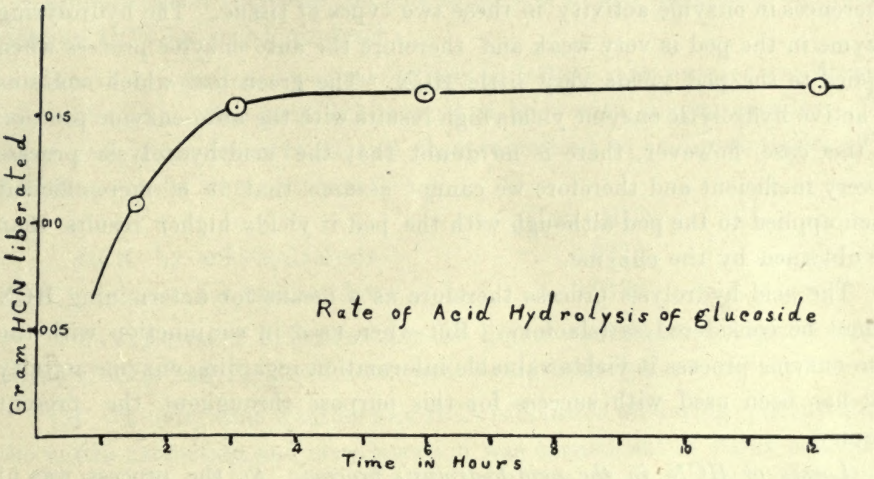

FIG. 1.

4. *Enzyme powder.* An extract having been prepared according to method (3) or other convenient procedure it was treated with enzyme powder and allowed to stand for 24 hours in a closed flask. The HCN liberated by the action of the added enzyme was then distilled off and determined as in the first method.

For the determination of HCN in the seed and plant parts of the Burma bean in almost every instance at least two of the above methods were employed.

It may be said at once that concordant results were never obtained; but a certain regularity in the discrepancies revealed itself at an early stage of the work, and this regularity in the discrepancies eventually explained some of the difficulties, and called attention to the need for a study of enzyme activity.

It was found for example that when dealing with fresh leaf the figure obtained by the auto-enzyme process was invariably higher than that obtained by the complete acid-hydrolysis method (including free HCN).

On the other hand determination of HCN in the green pod gave equally regularly the opposite result (namely, a low auto-enzyme figure and a high acid-hydrolysis figure).

It is unnecessary to quote data here. Typical examples will be found in most of the subsequent tables of analysis.

The peculiar results described above are now known to be due to differences in enzyme activity in these two types of tissue. The hydrolysing enzyme in the pod is very weak and therefore the auto-enzyme process when applied to the pod yields very little HCN. The green leaf which contains an active hydrolytic enzyme yields high results with the auto-enzyme process. In this case, however, there is no doubt that the acid-hydrolysis process is very inefficient and therefore we cannot assume that it is more efficient when applied to the pod although with the pod it yields higher results than are obtained by the enzyme.

The acid-hydrolysis process therefore as a means for determining HCN cannot be considered satisfactory. But when used in conjunction with the auto-enzyme process it yields valuable information regarding enzyme activity and has been used with success for this purpose throughout the present enquiry.

Losses of HCN in the acid-hydrolysis process. As the process was of value a number of experiments were carried out to determine where and how the losses involved in it occur. These experiments will be discussed next :—

(1) A crude glucoside extract prepared from leaf was divided into two parts, one part was submitted to acid-hydrolysis (method 3) and the other treated with enzyme powder (method 4). The results obtained were as follows :—

	Grm. HCN
HCN by acid-hydrolysis ..	.. 0·0038
„ enzyme powder ..	.. 0·0033

The test was made on several occasions but did not yield uniform results ; one test, for example, gave the following result :—

	Grm. HCN
HCN by acid-hydrolysis ..	.. 0·0056
„ enzyme powder ..	.. 0·0074

The efficiency of the enzyme powders could not be doubted. The lack of uniformity in the results could only be attributed to differences in behaviour of the different glucoside preparations.

(2) Losses which might occur during concentration of the aqueous extract preparatory to acid-hydrolysis were looked for.

An extract of crude glucoside was divided into four parts. Two of these were hydrolysed at once. The other two were diluted to double their volume, again concentrated down to their former volume and hydrolysed.

The four determinations yielded absolutely identical results.

Concentration therefore did not cause loss of any substance capable of yielding HCN by acid-hydrolysis.

Another experiment was carried out as follows: An extract was divided into two parts. One part was treated at once with enzyme powder. The other part was concentrated and then submitted to acid-hydrolysis. The following results were obtained :—

	Grm. HCN
HCN by acid-hydrolysis ..	.. 0·0076
,, enzyme powder ..	.. 0·0120

This experiment indicates that the liquor contains a substance which yields HCN to enzyme powder but not to acid-hydrolysis.

(3) To determine whether any HCN is distilled off during concentration, the entire extraction and concentration was carried out in flasks connected to condensers. The distillates were collected fractionally and examined for HCN. No trace of HCN was found in the distillates.

Loss of HCN was nevertheless proved to have taken place in this experiment. The original leaf contained 0·0270 per cent. HCN. Extraction and treatment of the extract with enzyme powder (to avoid all suspicion of loss through inefficiency of acid-hydrolysis) yielded 0·0195 per cent. HCN. Some free HCN was evolved at the commencement of the experiment. This amounted to 0·001 per cent. HCN. Out of a total of 0·027 per cent., therefore, 0·006 per cent. remains unaccounted for.

The loss in this case is not serious. It may amount to half the HCN of the leaf when acid-hydrolysis instead of enzyme powder is used. The experiments had to stop at this stage.

As far as they go they indicate that part of the loss at least is due to the presence in the extract of a substance which cannot yield HCN by acid-hydrolysis, but is able to do so when treated with enzyme powder.

There remains an observation in connection with other work which may be suitably quoted here.

It refers to fresh leaf in which the acid-hydrolysis process compares most unfavourably with the auto-enzyme method.

On three separate occasions after the HCN had been liberated as completely as possible by the auto-enzyme process and distilled off by steam, the residual liquor was concentrated by boiling and treated by the acid-hydrolysis process. A small but determinable amount of HCN was obtained in each case (by precipitation as Prussian blue). That is to say, the small residues of glucoside remaining after a vigorous enzyme has ceased to act, can be easily detected and determined by the water extraction and acid-hydrolysis process. The delicacy of the process therefore cannot be doubted.

We have to conclude that when fresh leaf is plunged into boiling water a certain amount of glucoside is converted into a substance which cannot yield HCN by acid-hydrolysis.

The effect, though not identical with, bears a resemblance to the observations of Viehover Johns and Alsberg with other plants.

Finally some remarks regarding the fourth process are called for.

When the total HCN content of an organ has to be determined the process has undoubted advantages over the acid-hydrolysis method.

In the first place concentration of the extract is not necessary—thus avoiding possible loss in this way. It is not even necessary to separate the plant part from the extract. The enzyme powder may be added as soon as the enzymes present in the organ under examination have been killed. The last precaution is undoubtedly necessary at times. An example illustrating this point is given later.

That it is able to liberate HCN in some form which cannot be liberated by the acid process has already been noticed.

II. Enzyme transformation of the glucoside and of HCN during drying of the leaf.

A. Hydrolytic power of fresh and sun-dry leaf.

The evolution of the so-called free HCN which has already been referred to under the water extraction method became prominent during the preparation and study of enzyme powder for the fourth method of estimating HCN.

The dried powder before extraction with alcohol was submitted to the systematic tests made on all plant tissues (*viz.*, determination of free HCN ; acid-hydrol. HCN ; and auto-enzyme HCN). This dry leaf was found to contain :—

(*a*) less total HCN than the original.

(*b*) a much higher proportion of free HCN than the original.

The following figures are typical of such sun-dried material :—

TABLE I.

Free HCN from fresh and sun-dried leaf.

	Free HCN	HCN by acid-hydrol.	HCN by enzyme
Fresh leaf 36 grm. ..	0·0020	0·0088	0·0200
Corresponding sun-dried leaf. ..	0·0086	0·0066	0·0150

Ravenna (*Jour. Chem. Soc.*, 1912, A. 2, 798, etc.) who has carried out a large number of tests on the evolution of free HCN by leaves when plunged into boiling water considers the phenomenon to be due to enzyme action during the short period of heating up required to kill the enzyme. No doubt this is generally the case, but if it were so in the present instance our sun-dried leaf should yield an extraordinarily active enzyme, seeing that its production of free HCN is strikingly high when compared with the fresh leaf.

As the primary question at that time was to procure an active enzyme powder, experiments were undertaken to compare the hydrolytic powers of enzyme powder prepared from fresh and sun-dried leaf respectively.

The experiment was carried out by extracting the tissue with alcohol and immediately using the material thus obtained for hydrolysing a crude glucoside solution. To make allowance for any deleterious effect of the alcohol upon the enzyme a series of enzyme powders, receiving increasing alcoholic treatment, was prepared from identical tissue, and the activity of each sample determined. The following results were obtained :—

TABLE II.

Effect of sun-drying on the activity of the hydrolytic enzyme.

Alcohol treatment	Time of action	GRM. HCN LIBERATED BY	
		Fresh leaf enzyme *	Sun-dried leaf enzyme *
	Mts.		
12 hrs.	30	0·0040	0·0012
	60	0·0072	0·0054
24 hrs.	30	0·0052	0·0040
	60	0·0130	0·0030
36 hrs.	30	0·0058	0·0050
	60	0·0102	0·0076
48 hrs.	30	0·0026	0·0020
	60	0·0098	0·0062

* In all cases quantity of enzyme is equivalent to 12 grm. fresh leaf.

These tests show in the first place that sun-dry enzyme is not more active than fresh leaf enzyme.

The figures also prove in a remarkable manner that alcohol is not injurious up to a certain point. It appears in fact that by continued soaking in alcohol the activity of the enzyme increases, reaches a maximum and then diminishes.

As it was intended to use this enzyme freely a further experiment to prove this point was undertaken. The experiment was carried out exactly as before, in this case young and old leaves being compared. Results obtained were as follows :—

TABLE III.

Comparison of hydrolytic power of young and old leaves.

Alcohol treatment	Time of reaction	HCN LIBERATED BY ENZYME OF			
		YOUNG LEAF		OLD LEAF	
		Fresh	Sun-dry	Fresh	Sun-dry
	Mts.				
12 hrs.	30	0·0030	0·0034	0·0030	0·0010
	60	0·0066	0·0068	0·0076	0·0038
24 hrs.	30	0·0106	0·0044	0·0050	0·0018
	60	0·0132	0·0076	0·0092	0·0050
36 hrs.	30	0·0054	0·0038	0·0030	0·0030
	60	0·0102	0·0074	0·0070	0·0068

Leaf enzyme powder used in each test was equivalent to 12 grm. fresh leaf. There is little doubt that the above-noted effect of alcohol is real. The point may be of interest to workers on plant enzymes. The experiments have established that young leaf is somewhat more active than old leaf ; and fresh leaf more active than sun-dry leaf.

The greatly increased evolution of free HCN by sun-dried leaf is therefore not due to increased enzyme activity but to a partial decomposition of the glucoside during sun-drying. The form in which the decomposition product exists in the leaf before boiling out is not known. Further experiments on this free HCN will be described below.

B. Losses of HCN during sun-drying of leaves.

Loss of HCN from leaves of cyanogenetic plants during drying has frequently been remarked upon.

Ravenna and Tonegutti (*J. C. S.* 1910, A. 2, 884) found that the loss was much greater when leaves were dried slowly at the ordinary temperature than when dried at 130°F. They remarked also that no HCN is evolved during the process.

Couperot (*J. S. C. I.*, 1909, p. 219) working with *Sambucus* found that drying led to simultaneous loss of nitrate and HCN. If the plant was dried quickly by heating to 60°F. there appeared to be no loss.

That HCN is lost during the drying of the leaves of Burma bean has been noted above.

A series of jar experiments was now undertaken to determine whether HCN as such is emitted during the process.

The apparatus consisted of large specimen jars into which wire trays were fixed. The air inlet consisted of a glass tube passing through the lid to the bottom of the jar. The exit tube drew air from the top of the jar and was connected through two glass bead alkali scrubbers, in series, to the water pump.

The efficiency of the apparatus for carrying off and absorbing HCN was tested as follows :—

Two jars with separate connections and absorbing tubes having been fitted up, the air current was turned on. Into the first jar on each of four consecutive days 1 c.c. of standard KCN solution was dropped on to a sheet of acidified filter paper. Into the second jar 4 c.c. were added at once. The aspiration was carried out continuously for five days. At the end of this time the HCN in the scrubbers and jars was determined.

The following figures were obtained :—

TABLE IV.

Efficiency test of jar aspiration and absorption of HCN in scrubbers.

	1st experiment	2nd experiment
	Grm. HCN	Grm. HCN
1st scrubber	0·0056	0·0038
2nd scrubber	0·0000	Trace.
Residue in jar..	0·0006	0·0018*

* Some water got into this jar and prevented evaporation of HCN.

Therefore for a steady evolution of HCN the apparatus is perfectly satisfactory.

Leaf was next dried in these jars, and no trace of HCN was obtained in the scrubbers. The test showed that under the conditions of this experiment HCN is not evolved by drying leaves.

The dry leaf produced in this way was next examined.

It was found to yield a small amount of free HCN and none whatever by auto-enzyme action.

The product obtained by this drying process is therefore very peculiar and differs entirely in its properties from the sun-dried material. The lack of auto-enzyme effect may be contrasted with the figures for sun-dried leaf in Table I. As the material produced was so unlike that which was sought, the laboratory jar experiment was abandoned for the time being, and a new apparatus set up for drying leaf under conditions similar to those which produced the original sun-dry powder.

To accomplish this the leaves were placed on a cement floor exposed to full sunlight, and covered by large bell jars. The jars were sealed to the floor by means of a thick layer of bees-wax through which two tubes were passed as inlet and outlet pipes respectively.

With the rapid drying of the leaves under these conditions a powerful air current was necessary to prevent condensation of moisture on the jars. Four sets of scrubbers had to be employed in parallel to give a sufficient passage for the large volume of air aspirated during the experiment.

When the leaves looked dry the experiment was stopped and the absorbing liquid in the scrubbers examined. It was found to contain an appreciable amount of HCN.

In this experiment therefore the leaves during drying had undoubtedly evolved HCN.

The experiment was repeated using young leaves (vigorous enzyme) and older leaves (less vigorous enzyme) and gave the following results :—

TABLE V.

Evolution of HCN by sun-drying.

	Young leaf	Older leaf
	Grm.	Grm.
Weight of fresh leaf	30·0	30·0
Weight after 5 hrs. sun-drying under bell jars	6·9	6·2
HCN evolved and obtained in scrubbers	0·0065	0·0020
HCN content of dried leaf { Free HCN (by boiling)	0·0017	0·0015
HCN content of dried leaf { HCN by acid-hydrol.	0·0007	0·0020

The figures indicate that fresh leaves whether young or old invariably evolve HCN when sun-dried, and the quantity so evolved may amount to half the HCN content of the leaf. The rate of evolution evidently depends upon the initial activity of the enzyme—the young leaf evolving decidedly more than the older leaf.

It will be observed too that the glucoside (*i.e.*, HCN by acid-hydrolysis) in the young leaf has been almost completely exhausted during the process, whilst in the old leaf an appreciable amount remains unconsumed.

One experiment was made to determine the time taken for the initiation of this change in the leaf.

Two lots of leaf were placed under jars as usual and aspirated. The scrubbers were renewed at two-hour intervals and the HCN evolved during these two-hour intervals determined.

The following figures were obtained in the two tests :—

TABLE VI.

Rate of HCN evolution during sun-drying.

	A	B
	Grm.	Grm.
Weight of leaf used	15.0	15.0
HCN evolved during .. { 1st 2 hrs.	0·0003	0·0000
HCN evolved during .. { 2nd 2 „	0·0022	0·0017
HCN evolved during .. { 3rd 2 „	0·0012	0·0019
TOTAL ..	0·0037	0·0036
FINAL WEIGHT OF LEAF ..	3·2	3·2

The evolution takes about two hours to commence. In the one case it was evidently coming to an end in six hours. In the other case it is not possible to say.

Owing to the time occupied by the necessary preliminary preparations for the experiment more than six hours' sun-drying could not be given. Further changes were therefore looked for by treating sun-dry leaf to aspiration in jars in the laboratory as in the preliminary trial.

For this purpose 150 grm. of fresh leaf were sun-dried for six hours. Weight after completion of this drying was 45 grm. The sample was divided into two parts, one part being placed at once in an aspiration jar, the other part being used for analysis. Laboratory aspiration with dry air in this case yielded a large amount of HCN. Finally the aspirated leaf was analysed. The following results were obtained :—

TABLE VII.

Jar aspiration of sun-dry leaf.

		Jar aspiration	Immediate analysis
		Grm.	Grm.
Weight of sun-dry leaf used		22·5	22·5
Weight after 4 days' aspiration		15·4	..
HCN in scrubbers ..	1st period of 2 days	0·0065	..
	2nd period of 2 days	0·0000	..
HCN in leaf ..	Free	0·0029*	0·0053†
	Acid-hydrol.	0·0032*	0·0071†
	TOTAL HCN	0·0126	0·0124

* After the aspiration treatment.
† Initial.

In this case the HCN has been completely accounted for. It will be found on reference to other data that this is invariably the case with sun-dry leaf. This drying process apparently liberates all the HCN existing in a form liable to destruction by boiling water.

It may also be noticed here that some glucoside was hydrolysed during the aspiration (acid-hydrol. figure has been very materially reduced). The leaf apparently contained sufficient moisture for this enzyme reaction.

It is most probable therefore that much of the HCN evolved during this aspiration period was also liberated by enzyme action set in motion during sun-drying. Sun-drying therefore directs the vital processes towards liberation of free HCN. In addition to this, however, the high " free HCN " content of the sun-dry leaf shows that it contains bodies which decompose in boiling water with evolution of HCN in a way which the glucoside of the fresh leaf does not.

C. Slow air-drying of leaf.

HCN having been obtained so readily in the last experiment by the laboratory jar aspiration method, this process was once more applied to fresh leaf which, it will be remembered, had given a negative result in the preliminary test.

This time the experiment was carried out simultaneously with a blank empty jar to which HCN was added. At the end of four days a complete recovery of HCN from the blank was obtained and again no trace from the leaf. It is certain therefore that leaf dried under these conditions does not evolve HCN.

The leaf after this treatment was thoroughly examined. It yielded a small amount of free HCN but zero by the auto-enzyme process. The leaf therefore had lost practically all its HCN, though none was evolved.

The fact that the leaf yields a little free HCN but none by the auto-enzyme process is remarkable. It shows that even the small residue of free HCN it contains disappears during the auto-enzyme process.

Assuming that the leaf had lost its hydrolytic power it was next treated with a very active enzyme powder for determination of HCN by the fourth process described above.

The enzyme powders used in this work were all prepared by extracting fresh leaf with alcohol. They are never quite free of HCN and a blank determination is always made to allow for HCN in the enzyme powder.

The test with this jar-dried leaf was carried out in duplicate, two flasks containing leaf and enzyme powder, two flasks enzyme powder alone. The flasks with enzyme powder alone yielded the usual amount of Prussian blue, but the flasks with leaf and enzyme powder gave no trace of colour.

This unexpected result was the first indication that HCN is destroyed in the presence of slowly dried leaf.

The experiment may also be noted as a case in which the enzyme powder process fails unless the enzymes existing in the tissue to be examined are destroyed before addition of the enzyme powder.

The total HCN content of this leaf as determined by boiling and extraction was—

Free HCN	.. ..	trace.
HCN by acid-hydrol.	.. .. ..	trace.

The original HCN in the leaf had therefore disappeared almost completely and none whatever had been evolved as gas.

Under conditions of slow air-drying therefore the HCN of the leaf appears to undergo enzymic auto-digestion.

A quantity of leaf was next slowly air-dried in the laboratory to determine whether such leaf possesses the power of destroying HCN. The following experiment was carried out with the material prepared in bulk:—

Into each of 8 flasks 5 c.c. KCN solution were measured. The liquids were made up to 50 c.c. in four of these and to 200 c.c. in the others. Six of the flasks were each treated with 4 grm. of leaf powder. Two flasks were treated with leaf powder previously boiled in water. The contents were then mixed, the flasks closed and allowed to stand overnight. Next morning the HCN was distilled off and determined as usual. The following results were obtained:—

TABLE VIII.

Destruction of HCN by slowly dried leaf.

	Contents of flask	Grm. HCN obtained
1.	Four grm. leaf and 45 c.c. water and 5 c.c. KCN	0·0020
2.	Ditto	0·0020
3.	Ditto leaf boiled	0·0064
4.	Four grm. leaf and 195 c.c. water and 5 c.c. KCN	0·0020
5.	Ditto	0·0020
6.	Ditto leaf boiled	0·0070
7.	Five c.c. KCN det. in concentrated solution without distillation	0·0090
8.	Ditto	0·0088

In all cases the leaf powder led to loss of HCN. The boiled leaf caused some loss, part of which is attributable to unavoidable loss of a trace in distillation.

It should be noted too that boiling of the leaf drives off the small amount of free HCN it contains. The flasks of boiled leaf therefore began with 0·002 grm. less HCN than the other flasks.

Destruction of HCN by unboiled leaf is unmistakably shown by these figures. The leaf itself was submitted to the systematic tests and yielded the following figures :—

	Grm.
Free HCN	0·0022
Acid-hydrol.	0·0008
Auto-enzyme	0·0000

The leaf shows again the typical effect of destroying the small amount of free HCN it contains when the auto-enzyme process is attempted with it.

The above experiment was next carried out in a modified form to determine the effect on glucoside as well as on HCN. Three flasks containing HCN and 3 flasks containing glucoside were used for the test. The experiment was carried out exactly as above with this difference that after distilling off the HCN as usual the residual liquor was submitted to acid-hydrolysis to determine any residual glucosidic HCN. The following results were obtained :—

TABLE IX.

Destruction of free and glucosidic HCN by slowly dried leaf.

	Grm. HCN by dist.	Grm. residual glucosidic HCN
1. Leaf powder + 5 c.c. KCN	0·0028	0·0008
2. Ditto	0·0024	0·0008
3. Boiled leaf + 5 c.c. KCN	0·0064	0·0010
4. Leaf powder + glucoside	0·0022	0·0008
5. Ditto	0·0022	0·0008
6. Boiled leaf + glucoside	0·0004	0·0054

The experiment corroborates the previous results and shows that the properties of the leaf, which cause it to lose the HCN it contains, persist in the dry leaf and are able to cause destruction of HCN both free and glucosidic.

The boiled leaf powder naturally cannot in any way affect the glucoside. The fact that HCN was still present in this case was revealed by acid-hydrolysis.

The experiment is striking, and if the determinations are made by precipitating the HCN as Prussian blue, gives a convincing ocular demonstration.

It may be noted that this slowly-dried leaf of the Burma bean behaves in a manner similar to the fresh tissue of *Tridens flavus* and other plants examined by Viehover Johns and Alsberg (*loc. cit.*).

D. Effect of fresh leaves on HCN.

Up to this stage the fresh leaf has never shown any indication of a destructive effect on HCN, but in view of the last result and the experience of workers with other plants it seemed desirable to carry out a more careful examination of this point.

The test was made as follows :—

Three solutions containing respectively 0·2286, 0·1740, 0·0870 grm. HCN per 1000 c.c. were prepared. Lots of 200 c.c. from each of these solutions were placed in bottles, and one lot of bottles with 200 c.c. water only was prepared. The bottles were brought to constant temperature in a bath maintained at 30°C. 30 grm. of fresh leaf were introduced into each bottle and the bottles replaced in the bath. The contents of the bottles were shaken at regular intervals. At the expiry of 2, 4 and 8 hours one bottle of each series was taken out, the liquid decanted, made alkaline to stop enzyme action and an aliquot heated, acidified and distilled. The HCN in the distillate was determined by iodine titration. The figure thus obtained includes the HCN originally added and that obtained from the leaf. By subtracting the initial HCN content of the liquor the HCN derived from the leaf is obtained.

In this way the HCN liberated from the leaf during each period was determined. The results obtained were as follows :—

Table X.

Effect of HCN solutions on the evolution of HCN by fresh leaf.

		A	B	C	D
Initial HCN content of soln. in grm.		0·04572	0·03480	0·0174	0·0000
HCN in grm. evolved by leaf during	1st period 2 hours	0·00003	0·00117	0·01124	0·00900
	2nd period 2 hours	0·01545	0·01770	0·00742	0·01110
	3rd period 4 hours	0·00780	0·01020	0·01020	0·01770
Total HCN evolved by leaf in grm.		0·02328	0·02907	0·02886	0·03780

The initial HCN content of the liquor is seen from these figures to have a marked effect upon the rate of evolution of HCN by the leaf. The total HCN evolved during the period is similarly influenced. The figures do not tell us

however whether the reaction had come to an end. To settle this point another set of determinations had to be undertaken. It yielded the following results :—

TABLE XI.

Effect of HCN solutions on the evolution of HCN by fresh leaf.

Initial HCN content of solution in grm.	A. 0·03396		B. 0·00000	
	Total HCN	HCN from leaf	Total HCN	HCN from leaf
Grm. HCN after 2 hrs.	0·04116	0·00720	0·00742	0·00742
In sol. „ 4 hrs.	0·04792	0·01396	0·01935	0·01935
„ 8 hrs.	0·05355	0·01959	0·02520	0·02520
„ 16 hrs.	0·05200	0·01804	0·02430	0·02430
„ 24 hrs.	0·05290	0·01894	0·02430	0·02430

The leaf is not identical with that used above but evidently under such conditions HCN evolution ceases entirely within a period of 8 hours.

Knowing that the reaction ceases in 8 hours it is possible from the results of Table X to draw curves showing roughly the velocity of HCN evolution from the leaf. These are shown below in Fig. 2.

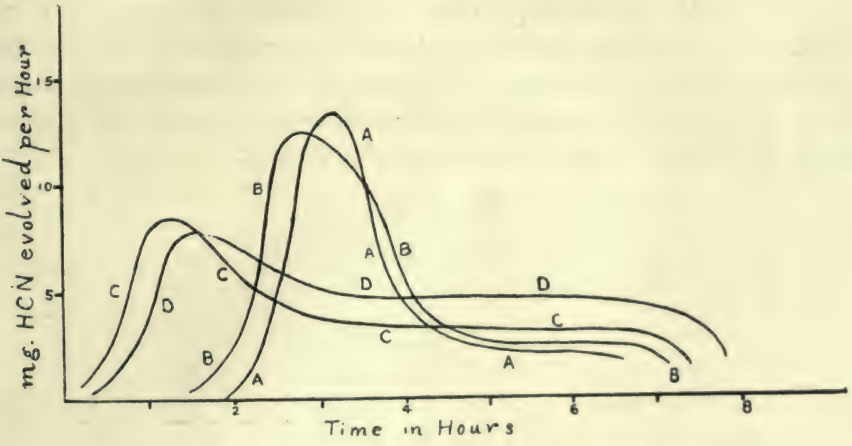

FIG 2. Showing velocity of HCN evolution.

Reference to the velocity graph brings out a number of points. In the first place it will be seen that when leaf is placed in relatively strong HCN solutions the evolution of HCN is very much retarded. It eventually comes out at a very great speed for a short period after which the velocity of evolution slowly dies down.

Another remarkable point brought out by this graph is that a weaker solution of HCN will accelerate the evolution—the initial speed in water being taken as our standard. The solution suitable to attain this result will no doubt depend upon the enzyme activity of the leaf. This is indicated by the fact that the results in the two tables from slightly different material, though similar, do not quite agree. From a combined examination of the graphs and the figures the following conclusions regarding this peculiar phenomenon may be arrived at :—

1. HCN in solution affects the permeability of the tissue. The permeability may be either increased or decreased according as the solution is weak or strong. The HCN in this case must play a part in regulating the vital processes in the leaf.
2. In the presence of HCN the hydrolysis ends prematurely. The total HCN obtained from the leaf being less in the presence of HCN than when water alone is used. This is apparently due to paralysis of the enzyme. This property of HCN must provide the plant with a delicate means for controlling hydrolysis.

The experiment does not reveal any definite sign of assimilation of HCN though it shows very clearly that the hydrolysis ends prematurely.

III. Enzyme activity of other plant parts.

In the young stalk the enzyme is sufficiently active to liberate large amounts of HCN by the auto-enzyme process. The usual test for enzyme activity, *viz.*, HCN determination by auto-enzyme action and by acid-hydrolysis gave in two samples the following results :—

TABLE XII.

Enzyme activity of young stalks.

	HCN FROM 36 GRM. FRESH STALK	
	By enzyme	By acid-hydrolysis
Sample 1	0·0054	0·0030
Sample 2	0·0078	0·0048

The auto-enzyme figure is decidedly higher than the figure by acid-hydrolysis which is taken as a proof of enzyme activity. This is not the case with all plant parts. The following table of analysis carried out on an early harvested crop shows the behaviour of various organs in this respect :—

TABLE XIII.

Analyses of crops harvested on 27th January, 1919.

Determination	HCN IN GRM. OBTAINED FROM 36 GRM. FRESHLY HARVESTED MATERIAL											
	Green leaf		Dead leaf		Stalk		Dry pod		Ripe seed		Green pod	
	Auto-enzyme	Acid-hydrol.	Auto-enzyme	Acid-hydrol.	Auto-enzyme	Acid-hydrol.	Auto-enzyme	Acid-hydrol.	Auto-enzyme	Acid-hydrol.	Auto-enzyme	Acid-hydrol.
Sample A, good seed ...	0·0136	0·0094	0·0010	0·0024	0·0076	0 0058	0·0010	0 0122	0·0000	0·0026	0·0016	0·0052
Sample B, bad seed ...	0·0076	0·0062	0·0006	0·0016	0·0036	0·0080	0·0004	0·0072	0·0016	0·0144	0·0002	0·0030

By good seed and bad seed is meant seed having low and high HCN content respectively.

The analyses were carried out immediately after the crop had been cut. The table contains a number of interesting points. It will be noticed for example that sample B is decidedly riper than sample A. The fresh leaves of B contain somewhat less HCN and the proportion of this recovered by acid-hydrolysis is higher than in A, which is a clear sign of decaying enzyme activity. These changes in the leaf have already been shown to accompany advancing maturity. The stalk too has advanced much further. It has lost much of its hydrolytic power—which is demonstrated again by the fact that the recovery of HCN by acid-hydrolysis is higher than by auto-enzyme action in sample B. The green pods are of considerable interest. They are the only part of the plant which in the perfectly green state is almost devoid of hydrolytic power—the amount of HCN liberated by auto-enzyme action being very small and forming also a very small proportion of the amount recoverable by acid-hydrolysis.

It was found that the pod begins to assume this character when the seeds commence to fill out. In December the young pods behaved like the leaves. There seems to be little change in HCN content of the pod during ripening. The relatively high figures for dry pod are mainly due to loss of moisture (as all figures in the table are based on 36 grm. of fresh sample).

By dead leaf is meant leaf which has become bleached and has just fallen.

In this case we are dealing with air-dry material comparable to the dry pods. The difference in HCN content of these two organs is striking.

The leaf during maturing (the figures for dead leaf should be divided by 4 to be compared with the fresh leaf) has lost almost all its HCN, whilst the pod has lost little or none. It is true this dead leaf gives higher HCN figures by acid-hydrolysis than by the auto-enzyme method, which proves that its hydrolytic power has been very much reduced. It is however still appreciable for the ratio of auto-enzyme HCN to acid hydrolysed HCN is not very low.

Judged by this criterion the hydrolytic power of the dry pod is negligible compared with the dead leaf.

Finally we come to the seed. The seed is also practically devoid of hydrolytic power and in this respect behaves similarly to the pod.

Concerning the fate of the HCN in the plant during ripening it may be concluded therefore that the leaf gradually parts with its HCN. Presumably this must pass in some form to the still vigorous parts of the plant.

A similar but less marked decrease takes place in the stem. The pod alone is peculiar in this respect, it does not discharge its HCN. It appeared possible from the results that the pod was assimilating N in the form of HCN. If so there was a possibility that it might show the phenomenon of auto-digestion.

Green and dry pods and dead leaf also were examined to test this point. The results of the test are given in—

TABLE XIV.

	Grm. HCN from 36 grm. fresh material		
	Old dead leaf	Late fresh husk	Green pod
Free HCN	0·0012	0·0003	0·0000
Acid-hydrol.	0·0050	0·0070	0·0050
TOTAL ..	0·0062	0·0073	0·0050
Enzyme HCN	0·0050	0·0006	0·0014
Followed by acid-hydrolysis of residue ..	0·0044	0·0066	0·0040
TOTAL ..	0·0094	0·0072	0·0054

There is no indication of assimilation in any of these cases. It should be explained that in this experiment no attempt was made to develop auto-

digestion power by slow drying or other means. The object was to determine whether the property was apparent in the organ in that state of development in which it was harvested.

We may note again that in the case of leaf—even of leaf which is virtually dead in this instance—there is an undetermined factor vitiating results. Here again the difference must be attributed to HCN compounds which are unstable in boiling water.

This process for discovering signs of assimilation was not applied to the seed ; but from what is now known of the seed it may be taken for granted that a negative result—similar to that observed in the case of the pod—would have been obtained.

A more refined test for auto-digestion has however since been applied to the seed. This will be described under seed experiments.

IV. Enzyme activity of seed in relation to age and storage conditions.

The study of enzyme activity in the plant has led to an important practical question. It supplied means for studying enzyme activity in the seed and also directed attention to this matter.

After the harvest experiments of January 1919 (given in Table XIII) had been completed the main facts relating to seed enzyme were very soon discovered.

In the results just referred to it was found, as already stated, that the hydrolytic enzyme of the harvested seed was very feeble in one sample and completely inactive in the other. But many seed samples had been examined prior to that time by the auto-enzyme process and had yielded HCN readily. This lack of enzyme power, therefore, seemed to be a property of fresh seed only.

Some samples of known origin and age were therefore procured from the bazaar and the test, which had now become a standard procedure for judging enzyme activity, was applied to these. The results obtained are given in Table XV.

Judging by the method previously employed, in every case the new seed was found to possess weak enzyme power and the old seed strong enzyme power. This enzyme activity has consequently been developed during storage.

TABLE XV.

Enzyme activity of new and old bazaar samples.

		HCN OBTAINED FROM 36 GRM. SEED BY		REMARKS
		Auto-enzyme process	Acid-hydrolysis	
New seed	1 ..	0·0072	0·0090	Enzyme HCN less than acid-hydrolysis, therefore weak enzyme.
	2 ..	0·0066	0·0094	
	3 ..	0·0012	0·0022	
Old seed	4 ..	0·0100	0·0084	Enzyme HCN more than acid-hydrolysis, therefore strong enzyme.
	5 ..	0·0100	0·0050	
	6 ..	0·0146	0·0089	

An experiment to study the effect of storage conditions on enzyme activity was therefore commenced.

For this purpose selected strains of seed were available. A strain having a high HCN content and one having a low HCN content were chosen for the work. The storage conditions tested were as follows :—

1. *Dry air ventilation.* To attain this, seeds were packed into a winchester bottle. Through the bung an air inlet tube was passed to the bottom and an exit tube attached to draw air from the top. 1000 c.c. of air bubbled slowly over H_2SO_4 were daily drawn through all bottles undergoing dry ventilation.

2. *Moist air ventilation.* The procedure was exactly as above with this difference that the air instead of being bubbled through H_2SO_4 was bubbled through water.

3. *Dry air unventilated.* Seeds were packed in desiccators with a beaker containing strong H_2SO_4 imbedded, up to its rim, in the seed.

4. *Moist air unventilated.* Seeds were packed into a winchester bottle, a definite amount of water added and the bottle sealed. The moisture contents of the seeds at the time of bottling was—

Sample B—high HCN content ... 8·375 per cent.
Sample A—low HCN content ... 8·115 per cent.

Sample B was bottled on the 23rd February and Sample A on the 12th March 1920. The table below shows weights of seed used for the experiment:—

TABLE XVI.

Weighings of storage samples.

Sample	Method of storage	Original weight	Final weight	Gain + Loss −	Date of opening
A. Good seed.	Dry ventilated ..	2487·2	2485·1	− 2·1	12–10–20
	Ditto ..	2241·8	2242·5	+ 0·7	9– 8–20
	Moist ventilated ..	2189·4	2187·7	− 1·7	14–10–20
	Ditto ..	2173·5	2175·7	+ 2·2	9– 8–20
	Dry unventilated ..	1145·4	1128·3	− 17·1	16– 8–20
	Ditto ..	1050·0	1028·5	− 21·5	18–10–20
	Moist unventilated ..	2148·4	2210·8	+ 62·4	16– 8–20
	Ditto ..	2189·9	2251·0	+ 61·1	20–10–20
B. Bad seed.	Dry ventilated ..	2444·4	2442·4	− 2·0	12–10–20
	Ditto ..	2225·0	2224·4	− 0·6	11–8–20
	Moist ventilated ..	2414·8	2417·8	+ 3·0	14–10–20
	Ditto ..	2397·9	2400·6	+ 2·7	11– 8–20
	Dry unventilated ..	1153·9	1134·6	− 19·3	18– 8–20
	Ditto ..	1090·9	1067·5	− 23·4	18–10–20
	Moist unventilated ..	2104·6	2166·4	+ 61·8	18– 8–20
	Ditto ..	2197·2	2257·7	+ 60·5	20–10–20

Certain points in these figures have to be noted. In the first place the data show that neither dry nor wet ventilation had any measurable effect on the moisture content of the seed. The vapour tension of the air used in these experiments was not determined. The air was simply bubbled through water in the one case and through H_2SO_4 in the other. To produce measurable changes of moisture content in this seed it is evident that the vapour tension must be made either much higher or much lower than was the case in these tests. The procedure, though it was quite ineffective as regards alteration of moisture content in the seed, was not without effect on the enzyme activity as will be seen later. The respiration of the seeds in these two tests was also examined for a time. No difference was found.

The wetted unventilated seeds had increased their moisture content from the initial 8·2 per cent to 10·7 per cent. This change in moisture content has produced marked effect on enzyme activity.

The seeds submitted to dry unventilated storage were slowly desiccated by the treatment. Their moisture content by the end of the experiment had fallen to 6·4 per cent.

The intention was to examine one sample of each series at intervals of six months. Only one such period was completed. Before the time for the next examination the writer had to leave ; but it was possible to complete a second set of determinations two months after the first.

The samples were examined with the greatest care, auto-enzyme determinations all being carried out in duplicate and the time factor in this process studied. Acid-hydrolysis was done in triplicate. As the results are of considerable significance the data are given in full detail in the accompanying table :—

TABLE XVII.

		Original fresh seed	First sampling				Second sampling			
			Dry ventilated	Moist ventilated	Dry unventilated	Moist unventilated	Dry ventilated	Moist ventilated	Dry unventilated	Moist unventilated
	Acid hydrolysis ..	0·0014	0·0008	0·0010	0·0014	0·0020	0·0026	0·0010	0·0014	9·0018
		0·0012	0·0009	0·0010	0·0016	0·0020	0·0024	0·0010	0·0014	0·0018
		..	0·0009	0·0010	..	0·0020	0·0024	..	0·0014	0·0018
A. Good seed. Auto-enzyme.	1 hr. ..	0·0002	0·0006	0·0006	0·0004	0·0008	0·0006	0·0006	0·0006	0·0012
		..	0·0006	0·0006	0·0004	0·0008	0·0006	0·0006	0·0006	0·0012
	2 hrs. ..	..	0·0006	0·0006	0·0006	0·0010	0·0014	0·0006	0·0006	0·0014
		..	0·0006	0·0006	0·0006	0·0010	0·0012	0·0006	0·0006	0·0014
	4 hrs. ..	0·0002	0·0004	0·0006	0·0006	0·0012	0·0016	0·0006	0·0006	0·0014
		..	0·0004	0·0006	0·0006	0·0012	0·0016	0·0006	0·0006	0·0014
	8 hrs. ..	..	0·0002	0·0006	0·0002	0·0006	0·0010	0·0004	0·0004	0·0012
		..	..	..	..	..	0·0010	0·0004	0·0004	0·0012
	24 hrs. ..	0·0006	0·0000	0·0006	0·0000	0·0010	0·0006	0·0004	0·0001	0·0012
		..	..	..	..	..	0·0006	0·0004	0·0001	0·0012

TABLE XVII—*concld*

		Original fresh seed	First sampling				Second sampling			
			Dry ventilated	Moist ventilated	Dry unventilated	Dry unventilated	Dry ventilated	Most ventilated	Dry unventilated	Moist unventilated
Acid hydrolysis ..		0·0138	0·0144	0·0146	0·0136	0·0140	0·0130	0·0136	0·0140	0·0134
		0·0140	0·0144	0·0144	0·0134	0·0142	0·0134	0·0140	0·0140	0·0136
		..	..	0·0148	..	..	0·0132	0·0140	0·0138	0·0132
B. Bad seed. Auto-enzyme. 1 hr. ..		0·0016	0·0056	0·0060	0·0054	0·0094	0·0066	0·0065	0·0056	0·0114
		..	0·0056	0·0060	0·0054	0·0094	0·0065	0·0066	0·0058	0·0114
2 hrs. ..		..	0·0060	0·0074	0·0054	0·0094	0·0064	0·0080	0·0057	0·0116
		..	0·0060	0·0074	0·0054	0·0094	0·0066	0·0079	0·0058	0·0116
4 hrs. ..		0·0022	0·0066	0·0074	0·0054	0·0094	0·0066	0·0074	0·0058	0·0116
		..	0·0066	0·0074	0·0054	0·0094	0·0066	0·0074	0·0058	0·0116
8 hrs. ..		..	0·0066	0·0074	0·0052	0·0094	0·0060	0·0074	0·0056	0·0110
		..	..	..	..	..	0·0060	0·0074	..	0·0110
24 hrs. ..		0·0060	0·0066	0·0074	0·0054	0·0094	0·0064	0·0068	0·0058	0·0102
		0·0064	..	..	..	..	0·0064	0·0070	0·0056	0·0106

The results obtained with the second test of dry ventilated sample B are given in full with the other figures. The results from this one bottle cannot be made to fit in with the other figures. They are inexplicable to the writer. From the remaining data certain points stand out quite clearly.

The difference between unventilated dry and wet storage is marked. In the dry storage the liberation of auto-enzyme HCN is restricted whilst in wet storage it is increased.

In comparing dry and wet ventilated storage we have only 3 sets of data to base our conclusions on. The differences produced by the two forms of treatment are slight ; but they are perfectly regular. In every case the moist ventilated sample, compared with the dry ventilated, shows a slightly increased production of HCN by the auto-enzyme process. This it should be remarked has taken place in spite of the fact that the moisture content of the seed has not been altered by an amount that could be measured (see Table XVI). The

result is of interest in showing that altogether imperceptible changes in the moisture conditions of the seed will cause perceptible enzyme changes.

A point which is quite definite also is that in every case after seven months' storage, even under the worst conditions, the acid-hydrolysis invariably yields higher figures than the auto-enzyme process.

Finally, with regard to the auto-enzyme process it is seen that the hydrolysis attains maximum efficiency within one hour, further action generally producing no increase whatever.

To bring out clearly the character of these stored samples one set of typical figures A, A, has been selected and tabulated together with results from typical bazaar seed in the accompanying table :—

TABLE XVIII.

Grm. HCN from 36 *grm. seed.*

	Fresh original A	Dry stored A	Bazaar fresh	Bazaar old	Bazaar fresh	Bazaar old
Acid hydrolysis ..	0·0139	0·0135	Not determined.	Not determined	0·0094	0·0050
Auto-enzyme 1 hr. ..	0·0016	0·0054	0·0070	0·0098	..	..
Auto-enzyme 2 hrs. ..	..	0·0054	0·0086	0·0124	..	..
Auto-enzyme 4 „ ..	0·0022	0·0054	0·0088	0·0124	..	..
Auto-enzyme 8 „ ..	..	0·0052	..	..	..	..
Auto-enzyme 24 „ ..	0·0062	0·0054	0·0096	0·0124	0·0066	0·0100

This table gives the characteristics of normal samples.

It will be seen that the auto-enzyme figures for our fresh and stored samples correspond fairly well with the typical fresh and old bazaar samples respectively. When auto-enzyme and acid-hydrolysis figures are both taken into account our fresh sample behaves normally. The acid-hydrolysis figure, as should be the case with fresh samples, is much higher than the auto-enzyme figure.

Our stored sample which coincided with the old bazaar sample when treated by the auto-enzyme process is quite different when the acid-hydrolysis figure is considered. Old seeds invariably yield a higher figure by auto-enzyme than by acid-hydrolysis, whilst our stored samples give without exception the opposite result.

We may say therefore that according to one test the stored seeds are old, according to the other test they are not old. We may conclude for the present that while they have changed in character to some extent during storage they have not yet advanced to the stage of being called old.

The storage results must also be compared with figures for seeds which were exposed freely to the air in a room by being spread in a single layer on wicker baskets. These seeds gave the following results :—

TABLE XIX.

Analysis of samples stored on trays.

	FEBRUARY		APRIL		JUNE	
	Auto-enzyme	Acid hydrol.	Auto-enzyme	Acid hydrol.	Auto-enzyme	Acid hydrol.
Sample A ..	0·0000	0·0026	0·0007	0·0026	0·0010	0·0010
Sample B ..	0·0016	0·0144	0·0036	0·0085	0·0120	0·0090

These figures show that seed thus freely exposed assumes the normal character of an old seed within four months. Our stored samples were still very far from this stage after six months' storage. The experiments indicate that storage under all the conditions tested restricts vital changes.

It is possible that conditions approaching the storage tests may occur in very large heaps. In any case the conditions existing in such heaps or in sacks could evidently be gauged by examining the seeds and comparing the results with the figures given above.

In small bazaar lots the conditions no doubt approximate to our trays.

The hydrolytic power of stored seeds. Referring again to Table XVII it will be seen that HCN determined by auto-enzyme action is higher after storage than it was in the original fresh seed. This should be an index of increased hydrolytic power.

It has already been observed also that this auto-enzyme liberation of HCN in the stored seeds is completed within one hour. Further action does not increase the yield. This would lead us to the belief that the hydrolytic enzyme is present in a very active form in these seeds.

Turning, however, to the acid-hydrolysis figures we find these are very much higher than the auto-enzyme results. The latter process therefore, although it comes to an end in one hour, is very far indeed from complete. A process which comes to an end in less than one hour and is so far from complete can scarcely be considered a normal enzyme hydrolysis.

Auto-digestion of HCN in the seed. Table XVII shows not only that maximum hydrolysis is attained very quickly but that further enzyme action leads in some cases to actual diminution of the HCN liberated.

We are here dealing with small quantities it is true but the precision with which the figures repeat themselves confirms the indications they give.

From previous work we know what the significance of this decrease is. The seed during the slow-drying process has developed the power of auto-digestion. The test is a very delicate one and may be found useful in future work. The effect of this auto-digestion on the yield of HCN by the enzyme process cannot be lost sight of. It no doubt partly accounts for the fact that the HCN liberated does not increase beyond a certain amount.

We have to conclude that the quantity of HCN liberated is the resultant of a series of balancing processes.

With regard to normal old seed a word must be added. Determinations of hydrolytic power of old seeds were carried out and the seed powder found to contain an active hydrolytic enzyme. Old seeds also invariably yield higher HCN figures by the auto-enzyme process than by acid-hydrolysis.

The above results with stored seed however cast some doubt on the efficiency of the auto-enzyme process with old seed even.

HCN content of seeds. The two cultures of beans used for the storage experiments described above were selected by the writer in 1915 as good and bad respectively. They have been grown regularly every year since then and each culture has maintained its characteristics perfectly.

It should be mentioned that the difference in HCN content is apparent only in the seed. By all the tests which are at present available the plants cannot be distinguished from one another. Both are full of HCN. The low HCN content of the good seed is therefore significant.

The plant has the property of producing seed in which the enzyme activities are so balanced that the HCN content remains slow. Considering the great difference between these two cultures in this respect it does not seem impossible that yet another culture should exist in which the balance is maintained at so low a level that HCN cannot be traced.

SUMMARY.

The chief points brought out during the course of this work may be summarized as follows :—

1. On plunging fresh leaves into boiling water the glucoside is hydrolysed to a considerable extent and part of the HCN is converted into a form which is not recoverable by acid hydrolysis. Similar but not identical effects have been recorded with other plants.

2. *The effect of drying the leaf.* It has been found that there is a fundamental difference between sun-drying and slow-drying. During sun-drying hydrolysis takes place with evolution of HCN. The residual dry leaf when plunged into boiling water evolves further large amounts of free HCN—an effect which fresh leaf does not produce.

   Another peculiarity of this sun-dry leaf is that it continues to evolve free HCN when treated by dry air aspiration. It was shown that this is probably due to a continuance of enzyme action.

   The amount of HCN evolved by sun-drying depends upon the initial enzyme activity of the leaf. Sun-drying directs the vital processes towards liberation of HCN.

   Slow-drying also causes loss of HCN ; but in this case none is evolved, auto-digestion taking the place of evolution.

   The resulting dry tissue is able to destroy HCN as such or HCN as glucoside. The enzymic balance of this slowly dried material approaches that found to occur in fresh tissue of other plants.

3. The hydrolytic power of young leaf is greater than that of old leaf and fresh leaf is somewhat more active than sun-dried leaf.

4. HCN has been found to have a powerful effect on cell permeability in the fresh leaf. It appears therefore that HCN in this plant acts as a regulator or Hormone.

   The presence of HCN has also been shown to bring glucoside hydrolysis to an end prematurely. The effect, which is probably due to paralysis of the enzyme, must play an important part in regulating HCN liberation within the plant.

5. In the fresh green plant the hydrolytic enzyme is also active in the stalk ; but it becomes weak as the plant matures.

6. The green pod and young ripe seed are peculiar in possessing practically no hydrolytic power.

7. The seed is found to develop hydrolytic power as it grows older.

8. The rate of development of hydrolytic activity in the seed depends upon the method of storage. Fresh seeds kept in the open air develop full characteristics of old seed within four months. Samples stored in bottles were still very far from this stage after six months' storage.

9. The drying of seed during storage reveals the power it possesses for auto-digestion of HCN. It is doubtless owing to this property that certain cultures have seeds with low HCN content.

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